Despite the development of the dental industry, the prevalence of diseases of the hard tissues of the teeth does not decrease but increases at a progressive rate [1, 2]. In recent years, an extremely common phenomenon that accompanies many dental diseases is dental hypersensitivity or hyperesthesia, which is manifested by pain, discomfort and leads to deterioration of the patient’s quality of life [3, 4]. In many respects, this situation is connected with both the lack of preventive measures and the lack of professional medicinal products that can effectively protect teeth from the aggressive effects of external factors.

In the case of treatment of dental hyperesthesia, patients usually use gels, varnishes, mouth rinses, toothpastes, reducing the volume of dentin micropores by increasing the mineralization of hard tissues or of special sealants for closing dentinal tubules [5–8]. The main components of remineralizing mixtures are most commonly various compounds of calcium, phosphorus, and fluorine [9, 10].

Unfortunately, the use of fluorine does not give a permanent effect, which requires the repetition of courses. The latter circumstance increases the risk of intoxication with fluorine compounds, which can lead to darkening of tooth enamel and pathology of the skeletal and central nervous systems [11]. Therefore, the use of medicinal products with fluorine is not recommended for children and pregnant women. It is also known that the increased content of fluorine in the body can lead to fluorosis – a chronic disease that affects mainly tooth enamel [12, 13].

An effective alternative to fluorine and a widely known and used calcium phosphate material in dental practice is hydroxyapatite, which is considered a crystal-chemical analog of the mineral component of human skeletal tissues (including teeth) and is a safe, biocompatible, and biodegradable material [14–16].

However, many compounds that are part of dental drugs are not able to provide a stable therapeutic and prevention effect due to their low penetration into the hard tissues of the tooth. Reducing the size of hydroxyapatite to nano- and microstructured systems has significantly increased the activity of this substance due to its ability to penetrate into microscopic spaces
between enamel prisms, seal dentinal tubules, integrate into the crystal lattice, and promote the formation of hydroxyapatite crystals of tooth enamel [9, 10, 16]. Accordingly, due to this, the enamel surface is densified and restored and the sensitivity of the teeth is reduced.

In addition, some of the above dosage forms (e.g., gels, pastes, rinses) are characterized by low bioavailability due to their rapid dilution by saliva. Therefore, to ensure the effect, they must be taken often and for a long time. Since most patients are tuned for instant and long-term result, a rational dosage form for the treatment of hyperesthesia is a dental medicinal film, which is characterized by good adhesion and prolonged effect [17–19].

Given the above, the subject of our research was the development of dental medicinal films with micronized calcium hydroxyapatite under the trade name “Kalident Powder 100” (Kalichem, Italy) for the treatment of dental hyperesthesia and caries prevention. According to the literature, hydroxyapatite is a sparingly soluble substance, therefore the purpose of our work was the justification of the optimal method of its introduction into the dental film.

**Materials and methods**

The objects of the study were:

– calcium hydroxyapatite under the trade name «Kalident Powder 100» (Kalichem, Italy);

– dispersion medium: purified water, ethyl alcohol 96%, polysorbate 80, glycerol, polyethylene oxide-400 (PEO-400), propylene glycol (PG), sunflower oil, vaseline oil.

Studies of physicochemical characteristics were performed both for the substance «Kalident Powder 100» separately and for mixtures of calcium hydroxyapatite with a mono-solvent and their combinations (in a ratio of 1:1).

In order to predict the behavior of the substance particles in different dispersion medium, the following physicochemical characteristics were determined: disperse and diffraction analysis of particle size distribution, morphological description, linear dimensions, shape factor \( k \), volume coefficient \( a_v \), Martin \( D_m \) and Ferret \( D_f \) diameters, wettability [20, 21].

Determination of crystallographic characteristics of «Kalident Powder 100» powder particles was performed by optical microscopy [20] using a microscope Konus Academy (Italy), equipped with a camera DLT-Cam Basic 2MP, and a microscope with an eyepiece-micrometer Krüss MBL-2100 (Germany). The obtained images were processed using DLT-Cam Viewer software.

The shape factor was calculated by formula (1):

\[
k = \frac{W}{L}. \tag{1}
\]

where \( W \) is the particle width;

\( L \) is the particle length.

Particles are considered isodiametric if \( k \) is close to 1.

The volumetric particle shape factor is determined by formula (2):

\[
a_v = \frac{0.455 \cdot \frac{h}{d_n}}{\sqrt{\frac{l}{d_n}}}. \tag{2}
\]

where \( h \) is the particle thickness;

\( l \) is the particle length;

\( d_n \) is the projected particle diameter.

Depending on the obtained value of the volume coefficient, we can speak about the shape of the particle: if the index approaches 0.455 it is a sphere, 0.303 corresponds to a cube, 0.231 is a plate, 0.183 is a needle shape [22, 23].
To obtain photographs, the sample after thorough mixing was placed on a glass slide as a monolayer. Particle sizes were measured by observing individual fields of view. Particles were considered to be in the field of view if they were located on one of the halves of its boundaries. If particle sizes of the powder were presented in a wide range, the measurement of the crystallographic characteristics of the samples was performed at different magnifications, which is due to insufficient depth of field of the microscope lens and, accordingly, the inability to obtain a quality image. The number of measured particles (when using one magnification) or their estimated number (when using two or three magnifications) must be at least 625.

The particle size distribution of «Kalident Powder 100» was determined by laser diffraction on a Shimadzu SALD-2201 laser diffraction analyzer (Japan) using WingSALD-II software, version 2.1.0. To do this, 20 mg of the substance was placed in a 100 ml volumetric flask, added 50 ml of dispersion medium and mixed thoroughly until complete wetting of the substance. The obtained dispersion was homogenized using an ultrasonic bath with a power of 50–100 W for 3–5 min, after which the volume of the dispersion was adjusted to the mark, shaken thoroughly, and immediately sampled for measurement [24, 25].

The wettability of the substance «Kalident Powder 100» was determined by direct measurement of the angle of contact (Θ) of a stationary drop of a certain liquid with a solid surface [20]. Calcium hydroxyapatite powder was slightly compressed into a disk to form a flat surface. A drop of a suitable liquid of a certain volume (approximately 1 μl) was placed on a powder disk and the angle of contact was directly measured using a protractor. Measurements of the wetting edge angle were performed immediately after contact of the liquid medium with the powder and after 15 sec. The time of complete wetting of the sample with the test liquid was also measured. The method of tangential determination was used to calculate the wetting angle in degrees [26].

The research results are presented as an average value. Statistical analysis was performed using Student’s t-test. The value of $p < 0.05$ was taken as the level of significance [20].

Results and discussion

Given the poor solubility of «Kalident Powder 100» in aqueous and non-aqueous mediums [14, 27], it was decided to enter it to the composition of the developed dental drug by the type of suspension. Therefore, an important task was to study the particle size of the substance, as it will directly affect the surface area available for reaction with cells and the biological fluid of the oral cavity. The results of microscopic analysis of the substance «Kalident Powder 100» are shown in Fig. 1.

Fig. 1. Micrograph of the substance «Kalident Powder 100» (magnification $\times$ 150)
The results obtained (Fig. 1) allow to conclude that «Kalident Powder 100» is a fine powder capable of agglomeration, as evidenced by the difference in the flow of light through the particles. Agglomerates are various in volume, their surface is inhomogeneous, porous; the linear size is in the range from 1 to 100 μm.

According to the disperse analysis of particle size distribution (Fig. 2) it can be concluded that the substance of hydroxyapatite calcium is not subject to the law of standard normal distribution. There is no clear maximum in the diagram, there is a large area between the differential curve and the abscissa, which indicates the presence in the powder of an almost equal ratio of particles of all fractions. The results indicate its polydispersity and possible obtaining of dispersion with small particles.

![Differential particle distribution curve of «Kalident Powder 100» at microscopic examination](image)

The next step in our research was to determine the crystallographic characteristics of calcium hydroxyapatite with the addition of aqueous and non-aqueous liquids, which are approved for use in oral medicinal products.

The first step was to study mixtures of «Kalident Powder 100» with a mono-solvent, prepared in a ratio of 1:1. The results are shown in Fig. 3.

As can be seen from the results (Fig. 3), the powder «Kalident Powder 100» in different mediums is able to divide the agglomerated particles into smaller ones, as evidenced by the change in their linear dimensions. The addition of mineral oil slightly changes the size of the agglomerates and the uniformity of distribution in the field of view. Under the influence of sunflower oil, there is limited wetting of hydroxyapatite particles with their coalescence. The sample of «Kalident Powder 100» with PG is a heterogeneous mixture with variations in the linear dimensions of agglomerated non-wetted particles from 10 to 1 μm. The addition of solvents such as purified water, glycerol, ethanol 96%, PEO-400, polysorbate 80 promotes the formation of sols with a decrease in the linear particle size from 7 to 0.01 μm and uniform distribution in the field of view of the microscope. In this case, the addition of polysorbate 80 promotes maximum wetting of the particles of calcium hydroxyapatite powder. Therefore, according to the degree of liquids influence on the homogeneity of the «Kalident Powder 100» particles distribution solvents can be differentiated as follows: glycerol > PEO-400 > polysorbate 80 > purified water > ethyl alcohol 96% > PG > sunflower oil > vaseline oil.
Fig. 3. Micrographs of the substance «Kalident Powder 100» in different mediums:

- $a$ – purified water;
- $b$ – ethanol 96%;
- $c$ – polysorbate 80;
- $d$ – glycerol;
- $e$ – PEO-400;
- $f$ – PG;
- $g$ – sunflower oil;
- $h$ – vaseline oil
The wettability of the powder is one of the parameters of the reduction degree of surface tension, dispersion, sedimentation, coagulation, and other surface phenomena [26]. In this regard, there is a need to assess this parameter when choosing the optimal dispersion medium. The wetting edge angle for all samples was determined by the fixed drop method. The results are shown in Table 1.

**Table 1**

Indicators of wetting angle (Θ) of the substance «Kalident Powder 100» with liquids of different nature

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Wetting angle (Θ), °</th>
<th>Full wetting time, sec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>15 sec</td>
</tr>
<tr>
<td>Purified water</td>
<td>10.1 ± 0.3</td>
<td>0</td>
</tr>
<tr>
<td>Ethanol (96%)</td>
<td>12.0 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td>Glycerol</td>
<td>17.6 ± 0.3</td>
<td>14.3 ± 0.3</td>
</tr>
<tr>
<td>PEO-400</td>
<td>25.0 ± 1.1</td>
<td>17.0 ± 0.9</td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td>12.0 ± 0.4</td>
<td>0</td>
</tr>
<tr>
<td>PG</td>
<td>37.2 ± 1.2</td>
<td>23.4 ± 1.1</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>49.3 ± 0.8</td>
<td>38.1 ± 1.5</td>
</tr>
<tr>
<td>Vaseline oil</td>
<td>51.6 ± 3.4</td>
<td>37.2 ± 0.9</td>
</tr>
</tbody>
</table>

Note: n = 3, p < 0.05.

The results obtained (Table 1) allow us to conclude that all the studied liquids wet calcium hydroxyapatite (Θ < 90°), but in different degrees: purified water > polysorbate 80 > ethanol 96% > glycerol > PEO-400 > PG > sunflower oil = vaseline oil. Liquids such as purified water, polysorbate 80 and ethanol 96% are the fastest to completely wet the surface of the sample (Θ = 0°). Therefore, given the results of microscopic analysis and determination of wettability of the substance «Kalident Powder 100», as well as taking into account the fact that the main solvent for the film-former in the manufacture of the dental film is purified water, we can conclude of the feasibility of further study of the effect of purified water in combination with PEO-400, glycerol and polysorbate 80.

The next step was to study the effect of a mixture of liquids on the ability of calcium hydroxyapatite to be distributed in their medium (Fig. 4). The solvent ratio was also 1:1. The study was performed immediately after the preparation of the mixture and a day later. At this stage, during the discussion of the results, not only the linear dimensions of the obtained particles were taken into account, but also the volume coefficient, their shape factor, Martin ($D_m$) and Ferret ($D_f$) diameters (Table 2).

**Table 2**

Crystallographic characteristics of a mixture of hydroxyapatite calcium (HAC) in two- and three-component mixtures of liquids

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No 1</td>
</tr>
<tr>
<td>$l$, μm</td>
<td>0.0110 ± 0.0003</td>
</tr>
<tr>
<td>$k$</td>
<td>0.99</td>
</tr>
<tr>
<td>$a$,</td>
<td>0.458</td>
</tr>
<tr>
<td>$D_m$, μm</td>
<td>0.013 ± 0.002</td>
</tr>
<tr>
<td>$D_f$, μm</td>
<td>0.00921 ± 0.00002</td>
</tr>
</tbody>
</table>

Note: n = 3, p < 0.05; $l$ – linear dimensions.
Fig. 4. Micrographs of the substance «Kalident Powder 100» in two- and three-component mixtures of liquids:

No 1 – water : polysorbate 80; No 2 – water : glycerol; No 3 – water : PEO-400; No 4 – water : PEO-400 : polysorbate 80; No 5 – water : glycerol : polysorbate-80

The results shown in Fig. 4, allow to conclude that the combination of purified water with polysorbate 80 (sample No 1), glycerol (sample No 2), and PEO-400 (sample No 3) contributes to a more homogeneous suspension of «Kalident Powder 100» due to
the decomposition of agglomerates into smaller particles as compared to the systems of the investigated powder with monosolvents. The smallest particle sizes of calcium hydroxyapatite with minimal deviation from the mean and their best distribution in the liquid medium are demonstrated by samples No 1, No 2, No 4, and No 5, the powder particles of which, according to the Table 2, are as close as possible to the spherical shape, as evidenced by the values of the volume coefficient (0.458, 0.449, 0.450 and 0.456, respectively) and the shape factor (0.99, 0.98, 0.97 and 0.91, respectively). This, in turn, allows predicting a uniform movement speed of powder particles and the absence of their adhesion when penetrating between the layers of the film former. Comparison of the obtained values of the Ferret and Martin diameters of substance «Kalident Powder 100» confirms the fact of uniform distribution of its particles by size in obtaining an aqueous suspension of calcium hydroxyapatite with the addition of polysorbate 80, glycerol, or PEO-400.

To identify possible structural formations, microscopic examinations of the above samples were performed after 24 hours of observation. The obtained results allow drawing a conclusion about the stability of the studied systems of multicomponent mixtures, as evidenced by the invariance of their crystallographic parameters over time.

For the final choice of the dispersion medium, the method of laser diffraction was used (Fig. 5 and Table 3).

Table 3

<table>
<thead>
<tr>
<th>Sample number</th>
<th>D (μm) in fractions containing a fraction of the total number of particles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%</td>
</tr>
</tbody>
</table>

Note: D is the maximum particle size of «Kalident Powder 100» in different fractions, containing 10% to 90% of the particles of their total number in the test substance.

Fig. 5. Particle size distribution curves of «Kalident Powder 100» in different mediums:
1 – purified water; 2 – purified water : polysorbate 80;
3 – purified water : PEO-400 : polysorbate 80; 4 – purified water : glycerol : polysorbate 80
According to the results of Table 3, all samples are subject to the law of normal distribution. The smallest bulk size of calcium hydroxyapatite particles was observed in samples No 2 and No 4 (4.640 μm and 4.953 μm, respectively), in samples No 1 and No 3 the particle diameter of «Kalident Powder 100» was about 6 μm. That is, the addition of liquids such as polysorbate 80 and glycerol to the aqueous suspension of calcium hydroxyapatite has led to an improvement in the distribution of micronized particles of «Kalident Powder 100» with a decrease in their maximum diameter, which allowed a more homogeneous system.

Therefore, comparative crystallographic analysis of «Kalident Powder 100» samples has allowed us to conclude on the feasibility of the use of a combination of purified water, glycerol, polysorbate 80, and PEO-400 in further studies on the development of the drug in the form of a dental film.

The obtained results will be used in further research when substantiating the optimal method of introduction of calcium hydroxyapatite «Kalident Powder 100» into the composition of the dental medicinal film.

**Conclusion**

1. Given the literature, it has been established that a rational approach to remineralization of teeth, restoration of enamel microcracks, and reduction of dentin sensitivity is the use of a micronized form of calcium hydroxyapatite, the main components of which are calcium and phosphorus – elements responsible for mineralization, integrity, and hardness of teeth.

2. Crystallographic characteristics of calcium hydroxyapatite under the trade name «Kalident Powder 100» (Kalichem, Italy) have been determined. It has been established that this substance is a polydisperse matter capable of agglomeration, which may adversely affect the homogeneity of its distribution in a dental medicinal product.

3. Studies to investigate the effect of different dispersion medium on the physicochemical characteristics of calcium hydroxyapatite have shown, that the largest change in the shape, size, and distribution of particles were observed in mixtures of «Kalident Powder 100» with hydrophilic liquids.

4. It has been found that the combination of the investigated powder with combined solvent systems containing purified water, polysorbate 80, glycerol, and PEO-400, allows achieving uniform distribution of the powder throughout the sample with satisfactory shape and size.

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STUDY OF PHYSICOCHEMICAL CHARACTERISTICS OF CALCIUM HYDROXYAPATITE «KALIDENT POWDER 100» IN THE DEVELOPMENT OF DENTAL MEDICINE

Key words: calcium hydroxyapatite, dispersion medium, crystallographic characteristics, wettability, laser diffraction

ABSTRACT

Despite the development of the dental industry, the prevalence of dental hypersensitivity does not decrease but increases at a progressive rate. Given the above, the subject of our research was the development of dental medicinal films with micronized calcium hydroxyapatite under the trade name «Kalident Powder 100» (Kalichem, Italy) for the treatment of dental hypersensitivity and caries prevention. To establish the optimal method of calcium hydroxyapatite introduction into the dental film, it was necessary to study the influence of different dispersion mediums on the physicochemical characteristics of the substance under study.

Purified water, ethyl alcohol 96%, polysorbate 80, glycerol, polyethylene oxide-400, propylene glycol, sunflower oil, vaseline oil were used as dispersion medium in the studies. Research of physico-chemical characteristics was performed both for the substance «Kalident Powder 100» separately and for its mixtures with the studied liquids and their combinations (in a ratio of 1:1). The following physico-chemical characteristics have been determined: disperse and diffraction analysis of particle size distribution, morphological description, linear dimensions, shape factor, volume coefficient, Martin and Ferret diameters, wettability.

The results of crystallographic and disperse analysis have revealed the polydispersity of the substance «Kalident Powder 100» and its high ability to agglomerate, which may adversely affect the homogeneity of its distribution in the dental medicinal product. A study of the effect of different dispersion medium on the physicochemical characteristics of calcium hydroxyapatite has shown a positive effect of hydrophilic liquids on the wettability of powder particles, changes in their shape, size, and distribution in the studied samples. It has been established that the combination of the substance «Kalident Powder 100» with combined solvent systems containing purified water, polysorbate 80, glycerol, and PEO-400, allows achieving optimal shape and particle size of calcium hydroxyapatite with their uniform distribution throughout the liquid, which is also confirmed by the results of laser diffraction. The obtained results will be used in further research on the development of dental medicinal film.
показало позитивний вплив гідрофільних рідин на змочуваність частинок порошку, зміну їх форми, розміру і розподілу у досліджуваних виразках. Встановлено, що поєднання субстанції «Kalident Powder 100» із комбінованими системами розчинників, що містять воду очищену, полісорбат 80, глицерин та ПЕО-400, дає змогу досягти оптимальних показників форми та розміру частинок гідроксиапатиту кальцію з їх рівномірним розподілом у всьому об’ємі рідини, що також підтверджено результатами лазерної дифракції. Одержані результати будуть використані у подальших дослідженнях із розроблення дентальної лікарської пляшки.

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ІССЛЕДОВАНИЕ ФИЗИКО-ХИМИЧЕСКИХ ХАРАКТЕРИСТИК ГИДРОКСИАПАТИТА КАЛЬЦИЯ «KALIDENT POWDER 100» ПРИ РАЗРАБОТКЕ СТОМАТОЛОГИЧЕСКОГО ЛЕКАРСТВЕННОГО СРЕДСТВА

Ключевые слова: гидроксиапатит кальция, дисперсионная среда, кристаллографические характеристики, смачиваемость, лазерная дифракция

АННОТАЦИЯ

Несмотря на развитие стоматологической отрасли, распространенность гиперчувствительности зубов не снижается, а растет прогрессирующими темпами. Предметом исследований стала разработка дентальных лекарственных пленок с микронизированным гидроксиапатитом кальция под торговым названием «Kalident Powder 100» (Kalichem, Италия) для лечения гиперестезии дентина и профилактики кариеса. Для установления оптимального способа введения гидроксиапатита кальция в состав дентальной пленки необходимым стало изучение влияния различных по природе дисперсных сред на физико-химические характеристики исследуемой субстанции.

Как дисперсионную среду в исследованиях использовали воду очищенную, спирт этиловый 96%, полисорбат 80, глицерин, полиэтиленоксид-400 (ПЭО-400), пропиленгликоль, масло подсолнечное, масло вазелиновое. Изучение физико-химических характеристик выполняли как для субстанции «Kalident Powder 100» отдельно, так и для ее смесей с исследуемыми жидкостями и их комбинациями (в соотношении 1:1). В работе определяли следующие физико-химические характеристики: дисперсный и дифракционный анализ распределения частиц по размерам, морфологическое описание, линейные размеры, фактор формы, объемный коэффициент, диаметры Мартина и Ферета, смачиваемость.

Полученные результаты были использованы в дальнейших исследованиях по разработке дентальной лекарственной пленки.

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